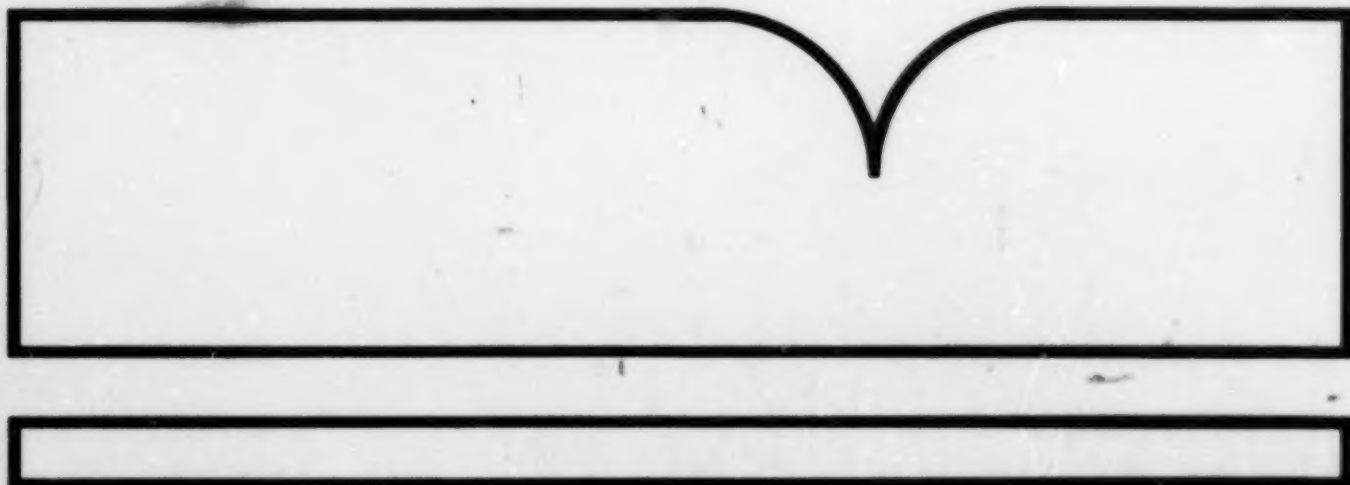


Chronic Ingestion of Cadmium and/or Tritium
II. Effects on Growth, Development, and
Reproductive Function

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Chronic Ingestion of Cadmium and/or Tritium

II. Effects on Growth, Development, and Reproductive Function

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The effects of chronic low-level exposure to cadmium (Cd) and/or tritium (HTO) on the growth and reproduction were evaluated in Sprague-Dawley rats. Cadmium exposure levels ranged from less than 0.1 to 5.0 ppm as CdCl₂ in the drinking water, while HTO concentrations ranged from less than 0.1 to 13.0 μ Ci/ml. Exposures were started with conception of the first generation. Both insults, at the higher doses, resulted in depressed adult body weight. Chronic Cd exposure caused liver weight decrements and a decrease in sperm counts. HTO effects were manifested in a depression of several reproductive parameters and brain weights in the F₂ neonates. Synergistic effects of simultaneous exposure were minimal.

INTRODUCTION

Simultaneous exposure to multiple environmental pollutants raises the question as to their possible synergistic action. Both Cd and HTO are ubiquitous components of the environment. Principal uses and environmental sources of Cd include electroplating and pigments, polyvinyl chloride stabilizer, and nickel-cadmium battery production (Minerals Yearbook, 1975; Technical and Microeconomic Analysis of Cadmium and Its Compounds). Future use in solar energy collectors may account for 50% of the Cd production. In addition to the above mentioned industrial sources of chronic Cd exposure, cigarette smoke has been shown to contain significant amounts of Cd (Scientific and Technical Assessment Report on Cadmium, 1975). Tritium is released to the environment mainly during reprocessing of spent fuel from nuclear reactors (Blomeke, 1964). Increasing release of tritium is anticipated in view of the steadily growing number of operating nuclear power plants.

Chronic exposure to either environmental pollutant has been shown to affect the life span and the reproductive function in laboratory animals (Schroeder *et al.*, 1965; Cahill *et al.*, 1970, 1975, 1976; Laskey *et al.*, 1973, Laskey and Bursian 1976). Effects on the central nervous system have been seen with acute doses of 0.7 mg/kg Cd (Gabbini, 1966; Gabbini *et al.*, 1967; Gunn and Gould, 1957) and with chronic exposure to HTO (Bursian *et al.*, 1975; Cahill *et al.*, 1976; Laskey and Bursian, 1976).

This study was designed to evaluate the individual and interactive bioeffects of chronic exposure of Sprague-Dawley rats to low levels of Cd and/or HTO from

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conception of the first generation (F_1) through delivery of a second generation. Concentrations of Cd and HTO employed were in low multiples of the established population exposure standards for each agent. For Cd the exposure concentrations were 10, 100, and 500 times the maximum permissible concentrations in water ($10 \mu\text{g/liter}$) (Federal Register, 1975) and for HTO 20, 200, and 2000 times the maximum permissible concentration in water (MPC_w) for continuous exposure of an individual in the general population ($0.005 \mu\text{Ci/ml}$) (ICRP No. 2, 1969).

A previous report (Pietrzak-Flis *et al.*, 1978) has shown that there is no alteration in the tissue accumulation of Cd with simultaneous exposure to HTO. This is a report of the bioeffects of chronic exposure to either Cd or HTO or to a combination of Cd and HTO.

MATERIALS AND METHODS

Sprague-Dawley (SD) male and female rats (Charles River Breeding Labs., Wilmington, MA) aged 90–100 days were mated. Sperm-positive females (P_0) were segregated into 10 groups: control, three Cd doses, three HTO activities, and three Cd-HTO exposures (9–10 rats in each group). Exposures were initiated at this point and continued throughout the experiment. Establishment of the initial body burden of HTO, segregation, housing, maintenance, and monitoring of equilibrium HTO levels in drinking water and urine were accomplished as previously described (Pietrzak-Flis *et al.*, 1978). Equilibrium HTO levels were maintained within $\pm 15\%$ of 0.10, 1.0, and $10.0 \mu\text{Ci/ml}$ of body water by allowing *ad libitum* access to drinking water with HTO concentrations of 0.13, 1.30, and $13.0 \mu\text{Ci/ml}$. Cd concentrations in the drinking water were 0.0, 0.1, 1.0, and 5.0 ppm. Cadmium drinking water solutions were prepared in deionized water from CdCl_2 (Fisher Scientific Company, certified ACS reagent). Periodic analysis of food indicated an average Cd content of 0.1 mg/kg of diet.

At term, four to five P_0 females from each dose group were delivered by Caesarean section. Term fetuses were removed after clamping off the umbilicus to prevent blood loss, litter size recorded, the fetuses dried, sexed, examined for gross malformations, and weighed. Two males and two females of known weight from each F_1 litter were dissected and the brain, lung, liver, and kidney removed and weighed. The remaining P_0 females delivered naturally and on Day 2 postpartum their F_1 litters were normalized to eight pups.

On Day 21 the pups were weaned and the P_0 dams sacrificed. At weaning and on Day 50, four to eight nonlittermate males and females from each dose group were weighed and sacrificed. Cardiac blood, brain, liver, kidney, and ovary or testes were removed and weighed. The epididymis was removed from each 50-day-old male and minced in 10% buffered formalin. Red blood cells (RBCs), white blood cells (WBCs), mean cell volume (MCV), hematocrit (Hct), and hemoglobin (Hgb) were determined from diluted blood samples using a Coulter Counter (Model ZB 1, Coulter Electronics, Inc., Hialeah, FL). Counting system was calibrated periodically with a 4C normal hematology reference control (Coulter Electronics Inc., Hialeah, FL). Total epididymal sperm of the 50-day-old males was determined on diluted aliquots of the minced epididymis using the Coulter Counter. Calibration

of the counter was performed by comparisons with visual counting using a hemocytometer.

At 100 ± 10 days, F_1 females were mated with nonlittermate males of the same dose group for 2 weeks. At term F_1 dams were delivered by Caesarean section and tissues were taken from both the dams and neonates as outlined above. Weighed F_1 males were sacrificed at 130 days of age and blood, brain, liver, kidney, testes, and epididymis were removed. Epididymal sperm were determined as previously outlined. Blood samples were taken from both selected adult males and term females and serum samples were prepared for analysis of selected hormones. Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were assayed by the radioimmunoassay (RIA) procedures (Midgley, 1966; Faiman and Ryan, 1967; Greenwood *et al.*, 1963; Odell *et al.*, 1967) using standards and antisera furnished by the National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health. Testosterone and progesterone were assayed by appropriate RIA procedures (Gupta *et al.*, 1972; Hillien *et al.*, 1973; Ismail *et al.*, 1972; Kinonchi *et al.*, 1973). Blood and tissue Cd content was determined by flameless atomic absorption and the results reported earlier (Pietrzak-Flis *et al.*, 1978).

The data were analyzed for Cd or HTO effects and for Cd-HTO interaction by a computer based two-way analysis of variance (ANOVA) system. The interaction effect was tested with the null hypothesis that the interaction was additive and linear. For each parameter analyzed, cell variances were tested for homogeneity (M. S. Bartlett, 1937). No significant deviation from homogeneity was found, therefore pooled standard deviations are given in the tables. Because of the effect of litter size on the number of resorptions and on mean litter weight, these data were evaluated by an analysis of covariance with litter size as the covariable.

RESULTS

No significant dose-related differences in body weight were seen in males during the first 100 days or in females at any point in the study. Body weights of males sacrificed at 130 days of age are shown in Table 1. Significant decreases in body

TABLE 1
BODY WEIGHTS (g) OF 130-DAY-OLD MALES^a

Treatment (ppm)	0.0 $\mu\text{Ci/ml}$	0.13 $\mu\text{Ci/ml}$	1.3 $\mu\text{Ci/ml}$	13.0 $\mu\text{Ci/ml}$
0	(9) ^b 502 ^c	(8) 526	(10) 470	(8) 485
0.1	(7) 491	(6) 416		
1.0	(9) 493		(9) 462	
5.0	(7) 480			(10) 453

^a Pooled standard deviation = 47. Overall ANOVA significant at $P < 0.01$. Cd effect significant at $P < 0.05$; HTO effect significant at $P < 0.05$, with no significant Cd-HTO interaction effect.

^b Sample mean.

^c Number of samples.

weight are seen due to Cd or HTO treatment and the decreases due to Cd-HTO treatment appear additive.

A 10 to 15% decrease in RBCs, Hct, and Hgb content was seen in 21-day-old male and female rats receiving both 5.0 ppm Cd and 13.0 $\mu\text{Ci/ml}$ HTO. No hematological effects were seen in other treatment groups at this age or in any group at other sacrifice times. No treatment effect was seen on WBC or MCV in any group at any sacrifice time.

Where treatment effects were found to be due to a single treatment regimen with no interactive effect, the data were pooled. Treatment effects found to be due only to chronic Cd exposure are presented in Table 2 and those due only to chronic HTO exposure in Table 3.

With the exception of the elevated relative liver weight in the 130-day-old F_1 males receiving 0.1 ppm Cd all other treatment parameters were depressed when compared to controls (Table 2). The majority of effects, where only chronic HTO administration produced effects (Table 3), were found in the highest exposure group. However, ovulations were depressed in F_1 term dams and sperm counts were reduced in 50-day-old F_1 males in both the 1.3 and 13.0 $\mu\text{Ci/ml}$ exposure groups (Table 3). No significant treatment effects were seen in relative liver weights in females at any of the sacrifice times. The relative liver weight of males was reduced by Cd treatment when measured at 50 days and 130 days of age (Table 2). No significant differences in relative liver weight were seen in males at any other sacrifice times.

The relative ovary weight of females sacrificed at 21 days was depressed approximately 30% in the 13 $\mu\text{Ci/ml}$ HTO exposure group. Combined exposure to HTO and Cd produced an interaction effect which was less than the effect of HTO

TABLE 2
EFFECTS DUE ONLY TO CHRONIC Cd EXPOSURE

	Cd exposure level (ppm)				Pooled SD	ANOVA <i>P</i> value
	0.0	0.1	1.0	5.0		
Liver weight (%)	(23) ^a	(5)	(10)	(15)		
50-Day-old F_1 males	4.4 ^b	4.4	3.9*	3.9*	0.5	0.01
Liver weight (%)	(34)	(11)	(17)	(19)		
130-Day-old F_1 males	2.76	2.80	2.65*	2.66*	0.23	0.01
Preimplantation deaths (avg/litter)	(37)	(16)	(14)	(16)		
F_1 females	1.85	1.57	1.44	0.82*	0.61	0.05
Serum progesterone (ng/ml)	(11)			(8)		
F_1 term females	6.07			4.53*	1.3	0.02
Kidney weight (%)	(27)	(17)	(12)	(16)		
F_1 male and female neonates (mean of litter means)	1.06	1.02	0.99	0.95*	0.12	0.01

^a Number of samples.

^b Sample mean.

* Significantly different from control value by Duncan's Multiple Range Test at $P < 0.05$.

TABLE 3
EFFECTS DUE ONLY TO CHRONIC HTO EXPOSURE

	HTO exposure level ($\mu\text{Ci/ml}$)				Pooled SD	ANOVA <i>P</i> value
	0.0	0.13	1.3	13.0		
Ovulations	(34) ^a	(15)	(14)	(20)		
F ₁ dams	15.5 ^b	14.6	13.7*	14.0*	1.64	0.01
Brain weights (%)	(16)	(8)	(6)	(6)		
F ₁ term males and females (mean of litter means)	3.75	3.85	3.90	3.53*	0.28	0.05
Brain weight (%)	(33)	(14)	(14)	(20)		
F ₂ term males and females (mean of litter means)	3.78	3.74	3.67	3.55*	0.21	0.01
Sperm count (number of epididymal sperm sperm/g testes $\times 10^{-3}$)	(23)	(6)	(9)	(13)		
50-day-old F ₁ males	3.82	3.97	2.33*	0.05*	0.44	0.05
Testes weight (%)						
21-Day-old F ₁ males	(16)	(8)	(8)	(10)		
	0.45	0.47	0.45	0.24*	0.19	0.01
50-Day-old F ₁ males	(24)	(7)	(10)	(13)		
	1.01	1.09	1.04	0.62*	0.21	0.01
130-Day-old F ₁ males	(32)	(14)	(19)	(18)		
	0.69	0.67	0.64	0.38*	0.18	0.01
Serum FSH-F ₁ males (130 days) (ng/ml)	(12)			(12)		
	295			456*	109	0.05
Serum LH-F ₁ males (130 days) (ng/ml)	(12)			(12)		
	184			264*	82	0.05

^a Number of samples.

^b Sample mean.

* Significantly different from control value by Duncan's Multiple Range Test at $P < 0.05$.

alone. The high variability, plus or minus 30%, makes this effect questionable. When examined at 50 days of age no differences in ovary weight were seen.

Exposure of the P₀ females had no effect on the measured reproductive parameters. Reproductive data on the F₁ females are presented in Tables 2, 3, 4, and 5. Ovulations were reduced by HTO treatment but were unaffected by Cd or Cd-HTO treatment (Table 3). Preimplantation deaths were reduced by Cd treatment and unaffected by the other treatments (Table 2). Resorptions were increased by HTO treatment (Table 4) and a greater than additive effect was seen with Cd-HTO treatment. Finally, litter size was decreased by HTO treatment, and the Cd-HTO treatment produced a greater decrease in litter size than was expected (Table 5).

At 21, 50, and 130 days of age relative testicular weight was depressed in the 13 $\mu\text{Ci/ml}$ HTO exposure group (Table 3) while Cd treatment did not produce observable effects. Combined Cd-HTO treatment produced no significant interaction effects on testes weight at any age.

Epididymal sperm content was evaluated at 50 and 130 days of age and is presented in Tables 3 and 6. In 50-day-old rats reduced sperm counts were seen in the HTO treated groups while no effect was seen due to Cd treatment and no

TABLE 4
RESORPTION IN F₁ FEMALES (AVERAGE %/LITTER)^a

Treatment (ppm)	0.0 μ Ci/ml	0.13 μ Ci/ml	1.3 μ Ci/ml	13.0 μ Ci/ml
0	(10) ^b 8.0 ^c	(9) 11.2	(7) 1.5	(10) 15.5*
0.1	(10) 7.5	(6) 3.8		
1.0	(8) 8.8		(6) 4.4	
5.0	(5) 7.1			(10) 28.4*

^a Pooled standard deviation = 7.0. Overall ANOVA is significant at $P < 0.01$. HTO effect and Cd-HTO interaction are significant at $P < 0.05$ and there is no significant Cd effect. Percentage resorptions adjusted for litter size.

^b Number of samples.

^c Sample mean.

interaction effect due to combined treatment with Cd and HTO. At 130 days of age a depression in sperm counts was seen with both HTO treatment and Cd treatment while Cd-HTO treatment did not cause a deviation from the additive linear hypothesis.

Serum levels of selected reproductive hormones in adult males and females are presented in Tables 2 and 3. Serum progesterone levels in term females were significantly reduced by Cd treatment with other treatments producing no significant effect. Serum testosterone levels were not significantly affected in any treated males when measured at 130 days. Serum LH and FSH levels were increased in male rats exposed to 13.0 μ Ci/ml of drinking water.

Selected observations on F₂ neonates are presented in Tables 2 and 3. Cd treatment resulted in a reduction in the relative kidney weight of F₁ neonates whereas no Cd effect was seen in the F₂ neonates. HTO treatment resulted in depressed relative brain weight in both F₁ and F₂ neonates but were unaffected by other treatments.

TABLE 5
AVERAGE LITTER SIZE IN F₁ FEMALES^a

Treatment (ppm)	0.0 μ Ci/ml	0.13 μ Ci/ml	1.3 μ Ci/ml	13.0 μ Ci/ml
0	(10) ^b 12.8 ^c	(9) 12.1	(8) 10.9	(10) 10.4*
0.1	(10) 12.9	(6) 12.7		
1.0	(8) 13.6		(6) 11.7	
5.0	(6) 14.8			(10) 10.0*

^a Pooled standard deviation = 2.23. Overall ANOVA significant at $P < 0.01$; HTO effect significant at $P < 0.01$; Cd-HTO interaction effect significant at $P < 0.05$ with no significant Cd effect.

^b Number of samples.

^c Sample mean.

TABLE 6
SPERM COUNT (NUMBER OF EPIDIDYMAL SPERM/g TESTES $\times 10^{-5}$) AT 130 DAYS^a

Treatment (ppm)	0.0 $\mu\text{Ci/ml}$	0.13 $\mu\text{Ci/ml}$	1.3 $\mu\text{Ci/ml}$	13.0 $\mu\text{Ci/ml}$
0	(8) ^b 2.19 ^c	(8) 2.00	(9) 1.88	(9) 1.02*
0.1	(7) 1.75	(6) 1.72		
1.0	(10) 1.97		(7) 1.69*	
5.0	(8) 1.68*			(10) 1.21*

^a Pooled standard deviation = 0.35×10^5 . Overall ANOVA was significant at $P < 0.01$, no Cd-HTO interaction effect; HTO effect significant at $P < 0.01$ and Cd effect significant at $P < 0.05$.

^b Number of samples.

^c Sample mean.

DISCUSSION

Table 7 summarizes the bioeffects of the individual and interactive effects found in this study.

The biological responses of the SD rat to chronic HTO exposure are consistent with previously reported results (Cahill and Yuile, 1970; Laskey *et al.*, 1973; Cahill *et al.*, 1976; Laskey and Bursian, 1976) in that the more sensitive indicators

TABLE 7
SUMMARY OF BIOEFFECTS

	CD	HTO	CD-HTO interaction
% Liver weight—males at 50 days	↓	None	None
% Liver weight—males at 130 days	↓	None	None
Preimplantation death—F ₁ dam	↓	None	None
Serum progesterone—F ₁ dam	↓	None	None
% Kidney weight—F ₁ pup male	↓	None	None
Body weight—males at 130 days	↓	↓	None
% Testis weight—males at 21 days	None	↓	None
% Testis weight—males at 50 days	None	↓	None
% Testis weight—males at 130 days	None	↓	None
Epididymal sperm count at 50 days	None	↓	None
Epididymal sperm count at 130 days	↓	↓	None
Serum FSH	None	↑	None
Serum LH	None	↑	None
Ovulations—F ₁ dams	None	↓	None
Litter size—F ₁ dams	None	↓	↓
Resorptions—F ₁ dams	None	↑	↑
% Brain weight—F ₁ pups	None	↓	None
% Brain weight—F ₂ pups	None	↓	None
RBCs at 21 days—F ₁ males and females	None	None	↓
Hct at 21 days—F ₁ males and females	None	None	↓
Hgb at 21 days—F ₁ males and females	None	None	↓

of chronic low-level HTO exposure are the reproductive and central nervous systems. An increase in resorptions with concurrent reduction in litter size is consistent with the data reported by both Cahill and Yuile (1970) and Laskey *et al.* (1973, 1976) following either the short-term chronic gestational HTO exposure or the lifetime exposure. Both these studies demonstrated dose-related decrements in brain weight. The testicular weight reduction and depressed epididymal sperm count seen with HTO exposure in this study support the data previously seen (Cahill and Yuile, 1970; Laskey *et al.*, 1973; Laskey and Bursian, 1976) from both the short-term chronic gestational exposure and by lifetime exposures. Elevated serum FSH was expected with a depressed sperm count (Means, 1977), and elevated pituitary FSH was found in the previous HTO study (Laskey and Bursian, 1976).

The responses to chronic Cd exposure were also consistent with published reports of the biological systems affected in both acute and chronic Cd exposure (Friberg *et al.*, 1974). According to the water consumption data (Pietrzak-Flis *et al.*, 1978), the F₁ rats received approximately 1.3 mg Cd/kg/day as young animals which decreased to 0.5 mg Cd/kg/day as they reach adulthood. The higher dose (1.3 mg/kg), if administered parenterally to adult animals, has been shown to result in testicular hemorrhagic necrosis with subsequent total testicular degeneration (Gunn *et al.*, 1968). Sutton (1939) did not find any effect on male fertility following chronic dietary exposure to 150 ppm Cd. Schroeder (1973) using chronic Cd exposure (10 ppm) via the drinking water found fertility unaffected in mice. Although we did not observe any gross testicular pathology or a depression in fertility, the epididymal serum content was reduced approximately 20% in the 5 ppm Cd group at 130 days. No increase in serum FSH accompanied the reduced sperm count in the group treated with 5 ppm Cd only but was seen in the group receiving both 5 ppm Cd and 13 μ Ci/ml HTO.

A Cd dose-related depression in the mean F₂ litter weight (adjusted for litter size) was found and is consistent with Schroeder's data showing an increase in the number of runts following chronic Cd (10 ppm) exposure in mice. Webster (1978) also demonstrated a depression in mean litter weight (adjusted for litter size) in mice whose mothers received as little as 10 ppm Cd in the drinking water throughout gestation.

The slight but significant depression in RBCs, Hgb, and Hct observed in 21-day-old weanlings receiving both 5 ppm Cd and 13 μ Ci/ml HTO is comparable with data presented by Webster (1978) where he saw a 25 to 50% depression in mice at term whose mothers received 40 ppm Cd during gestation.

The slight but significant reduction in percentage liver weight was not unexpected as others (Sporn *et al.*, 1970; Sugawara and Sugawara, 1978) have seen alterations in liver metabolic function following chronic 1 ppm Cd exposure.

All of the data gathered in this study were subjected to statistical analysis for interactive effects of simultaneous exposure to both Cd and HTO. In the majority of analysis a greater than or less than linear-additive effect was not seen, however, the 21-day-old blood parameters, the F₂ resorption rate, and litter size did show a greater than linear additive response to chronic Cd and HTO exposure. This study demonstrates that the biological effects of exposure to these compounds singularly could be used to predict bioeffects of their simultaneous exposure.

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